BIO-INORGANIC CHEMISTRY

Oxyhemoglobin  Deoxyhemoglobin

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BIO - INORGANIC CHEMISTRY

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Topics Covered

Chapter 1

Basic Introduction

1.1 Introduction:

Bio-Inorganic chemistry is a field that examines the role of metals in biology. It is the combination of biochemistry and inorganic chemistry. Many inorganic elements are essential for life. It comprises the study of both natural phenomena and behaviour of metalloproteins as well as artificially introduced metals, including those that are non-essential, in medicine and toxicology.

Example: Fe (heme) ------ Hemoglobin, Cu -------- Plastocyanin

Inorganic Elements In Biological Systems:

1. Living organisms store and transport transition metals (d-block elements).
2. To provide the appropriate concentrations in metalloproteins or co-factors and to protect themselves against the toxic effects of metal excesses.
3. Metalloproteins and metal co-factors are found in plants, animals and micro-organisms.
4. The normal concentration range for each metal in biological system is narrow. (i.e.) both metal deficiency and excesses will cause some pathological changes.
5. The form of metals is always ionic.
6. Decreasing order of transition metals present in biological systems are Fe, Zn, Cu, Mo, Co, Cr, V and Ni.
7. Iron is the most common transition metals in biology. The processes and reactions in which iron participates are essential to the survival of terrestrial organisms.
8. Ribo nucleotide reduction (DNA synthesis), energy production (respiration), energy conversion (photosynthesis), nitrogen reduction, oxygen transport (respiration), muscle contraction and oxygenation.

9. Among the transition metals used in living organisms. Iron is the most abundant metal in the environment.

**Role Of Alkali And Alkaline Earth Metals In Biological Systems:**

1. The alkali elements are essential for the normal functioning of bio-systems.
2. Concentration of some alkali and alkaline earth metal ions is in the following table.

Concentration (mmol/litre)

<table>
<thead>
<tr>
<th>System</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Cl⁻</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>11</td>
<td>92</td>
<td>10⁻⁴</td>
<td>2.5</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>BLOOD PLASMA</td>
<td>160</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>

3. **Role of Calcium:**
   - Calcium controls the mechanical stability of the walls of some cells. (e.g.)  
     Muscle contraction is present in living beings.
   - It also includes in fertilization, cell division and hormonal activities.

4. **Role of Magnesium:**
   - Magnesium ions are present in high concentration in biological systems.
   - Magnesium is vital in stabilizing DNA, RNA, proteins and lipids.

5. The different alkali and alkaline earth metal ion concentrations in the human body are as follows.
K$^+$ and Na$^+$ ----10$^{-1}$M, Mg$^{2+}$ and Ca$^{2+}$-------10$^{-3}$M

6. K$^+$ and Na$^+$ ions are the cations, present in high concentration than others.
Chapter 2

Bio-Inorganics in Living Systems

Transport Across Membrane:

1. Metal ions are transported across the membranes with molecular transport system.
2. There are two types of molecular transport system.
   a. Active transport
   b. Passive transport
3. Active transport means the movement of ion against the concentration gradient.
4. Passive transport is thermodynamically favoured.

Ionophores:

- Ionophores are macro molecular antibiotic substances capable of inducing the passage of specific cations across biological membranes.
- Molecular weight of the Ionophores is in the range of 500-2000 Daltons.
- Ionophores are classified based on their structures into neutral and carboxylic ionophores.
- When neutral Ionophores makes complexation with a metal ion, net cationic charge is associated with them.
- Carboxylic Ionophores contain a chain of covalent carbon to carbon bonds and a single terminal carboxylic group involved in head to tail ring closure with a hydrogen bonding.
Neutral Ionophores:

1. Cyclic Natural Ionophores:

It forms metal complexes with monovalent alkali metal ions it is divided into macrotetrolides and cyclodepsipeptides.

Macrotetrolides

1. Nonactin:

1. It is a typical macrotetrolides with a 32 membered ring structure.
2. Four carbonyl oxygen atoms and four tetrahydrofuran oxygen atoms bind the $\text{K}^+$ ion.
3. The cation is buried inside the hydrophilic central cavity of Nonactin.
4. The exterior of the ionophore contains many $\text{CH}_3$ groups on its periphery and so it is
5. lipophilic (or) hydrophobic.
6. Monactin has an additional $\text{CH}_2\text{-CH}_3$ group.

![Nonactin structure](image)

Where R=H; Nonactin, R= CH$_3$, Monactin

2. Valinomycin:

1. It is a cyclodepsipeptide.
2. It is involved in active transport of $K^+$.  
3. It consists of alternate residues of hydroxy acids and amino acids.  
4. Six of the amide carbonyl oxygen atoms form a three-dimensional cage around the metal ions.  
5. The oxygen atoms form a bracelet like structure of $4 \text{Å}^\circ$ height and $10 \text{Å}^\circ$ unit diameter.  
6. The available space at the centre of the ionophore decides the cation to be bound by it.  
7. $K^+$ ion with radius $1.33 \text{Å}^\circ$ which perfectly fits into the cavity of Valinomycin as that of $Na^+$ ion with $0.9 \text{Å}^\circ$.  
8. The stability constant $K$ for the potassium-valinomycin complex is larger than that of sodium-valinomycin complex, the difference is that important for maintaining the selectivity of valinomycin for the transport of potassium ions and not sodium ions in biological systems.

![Ionophore Structure](image)

3. Monensin:  
1. Monensin is a carboxylic acid ionophore isolated from streptomyces cinnamonensis.
2. This ionophore is related to the crown ethers with a preference to form complexes with monovalent cations such as Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), Ag\(^+\) and Ti\(^+\).

3. Monensin is able to transport these cations across the lipid membranes of the cells.

**Mechanism of ion transfer through membrane by ionophore:**

1. Ionophore is a substance that can transfer ions from a hydrophilic medium such as water, into a hydrophobic medium such as biological membrane.
2. Biological membrane is a lipid phase inserted in an aqueous medium.
3. Aquated cation is present in water.
4. Ionophores are low molecular weight natural products which dissolve in the plasma membrane or intracellular membranes of cells and make the membrane permeable to specific ions.
5. Once, the ionophore senses the presence of a cation a cycle of operations is initiated as shown in the following table.

Where I - refer the ionophore

MI+- cation-carrier complex

<table>
<thead>
<tr>
<th>SIDE 1</th>
<th>SIDE 2</th>
<th>SIDE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>((H_2O)_n M^+ \rightarrow I)</td>
<td>I (\rightarrow)</td>
<td>IM(^+) (\rightarrow) ((H_2O)_n)</td>
</tr>
<tr>
<td>((H_2O)_n M^+ I)</td>
<td>LIPOPHILIC BARRIER (M^+ I)</td>
<td>(M^+ (H_2O)_n)</td>
</tr>
<tr>
<td>(N (H_2O) \leftarrow M^+ I)</td>
<td>I</td>
<td>I (\rightarrow M^+ (H_2O)_n)</td>
</tr>
</tbody>
</table>
Different Steps Involved In The Process Are:

1. The first stage is the formation of complex between cation and ion carrier.
   a. In complex formation, the ion forms are co-ordination complex with the ionophore in which there is a well-defined ratio (typically 1:1) of ion to ionophore.
   b. The ionophore wraps around the ion so that the ion exists in the polar interior of the complex, while the exterior is predominantly hydrophobic in character.
   c. The ionophore molecule essentially acts as the solvent for the ion, replacing the aqueous salvation cell (H₂O molecule) that normally surrounds the ion.

2. In the second stage, diffusion of the cation-carrier complex through the membrane is carried out.

3. In the third stage, the cation is released. This step regulates the free ionophore for further cation transport.

Transport of K⁺ by Monensin ionophore is shown below. The transport of K⁺ from a lower concentration side to a higher concentration side (active transport) of Monensin is visualized as below. The process involves cation transport coupled with proton transport. Ionophores are a class of compounds that form complexes with specific ions and facilitate their transport across cell membranes. An ionophore typically has a hydrophilic pocket (or hole) that forms a binding site specific for a particular ion. Some, ionophores are synthesized by microorganisms to import ions into their cells.

\[
\text{Step-1: } K^+ (aq) + HI (Lipid) \leftrightarrow K^+I^- (Lipid) + H^+ (aq)
\]

\[
\text{Step-2: } K^+I^- (Lipid) + H^+ (aq) \leftrightarrow HI (Lipid) + K^+ (aq)
\]

Ion transport mainly depends on the lipophilicity of the resulting carrier complex.
Step-3:

Sodium-Potassium Pump: (Na⁺/K⁺ Pump)

1. The sodium potassium pump is a specialized type of transport protein found in your cell membranes. The cell membrane is the semi-permeable outer barrier of many cells.
2. The Na/K pump’s job is to move potassium ions into the cell while simultaneously moving sodium ions out of the cell. (The sodium potassium pump uses active transport to move molecules from a high concentration to a low concentration).
3. This pump is powered by ATP (Adenosine triphosphate). For each ATP that is broken down, 3 sodium ions move out and 2 potassium ions move in. In order to move the ions (Na⁺/K⁺) against their gradients, energy is needed. This energy is supplied by ATP.
4. Sodium ions bind to the pump and a phosphate group from ATP attaches to the pump, causing it to change its shape.
5. In this new shape, the pump releases the three sodium ions and now binds two potassium ions. Once the potassium ions are bound to the pump, the phosphate group detaches.

6. This in turn causes the pump to release the two potassium ions into the cytoplasm. The sodium-potassium pump is an anti-porter transport protein.

7. The sodium potassium pump is vital to numerous bodily processes, such as nerve cell signaling, heart contractions and kidney functions. The Na/K pump is a specialized type of transport protein found in your cell membranes.

8. The sodium–potassium pump is the integral in maintaining the acid-base balance as well as in healthy kidney function. Energy is derived from pumping sodium outside the cell, where it becomes concentrated, wanting to push its way back in. This energy is used to remove acid from the body.

9. The sodium-potassium pump is an important contributor to action potential produced by nerve cells.

10. This pump is called a P-type ion pump because the ATP interaction phosphorylates the transport protein and causes a change in its conformation.
**Metallo Porphyrins:**

Porphyrins are a group of heterocyclic macrocycle organic compounds composed of four pyrrole subunits with conjugated double bonds interconnected at their $\alpha$ carbon atoms via methane bridge (\(=\text{CH}\)).

1. It is the combination of metal ion with porphyrin ring.
2. In general, the porphyrins complex with dipositive metal ions to form metal porphyrin complexes.
3. The hole in the centre of the porphyrin ring is ideal for accommodating metals of the first transition series.
4. The porphyrin system is fairly rigid, because of the delocalization of the $\pi$ electrons in the pyrrole rings.
5. The metal (Ni) – N bond distance is approximately 193-196 pm in Ni porphyrin.
6. In Fe$^{2+}$ porphyrins the bond distance is about 2 pm (picometer).
7. If the size of the metal is too small, the ring becomes ruffled to allow closer approach of the nitrogen atoms to the metal.
8. If the size of the metal is too large, it cannot fit into the hole and since it is above the plane of the ring.

**Physical And Chemical Properties:**

1. Small variations in properties of the compound are possible by varying the substituents on the periphery.
2. Porphyrin is a good $\sigma$ donor and also an effective $\pi$ acceptor.
3. Fe can undergo redox reaction depending on the environment.
4. It is limited in water solubility especially with after bonding with 2 extra axial ligands and readily enters hydrophobic solvents.
5. Ion does not dissociate from heme so that heme ion is a new element distinct from ion.
6. Metal porphyrins are biologically accessible compounds can be varied by changing
   a. The metal
   b. oxidizing state
   c. The nature of the organic substituents and the
7. porphyrin structure.
8. The order of stability of metal porphyrins is Ni$^{2+} >$ Cu$^{2+} >$ Co$^{2+} >$ Fe$^{2+} >$ Zn$^{2+}$.

**Cytochromes:**

1. Cytochromes are a group of iron-heme proteins that functions as electron carriers in mitochondrial oxidation, photosynthesis, etc.,
2. They are classified as a, b, c etc.,
3. Cytochromes a, b & c differ in their porphyrin substitution and in iron coordination (CoNH2) amide group.
4. Cytochrome (A) has a formyl group (CHO) group at position 8 of the porphyrin ring and has a long hydrocarbon chain C$_{17}$H$_{29}$O at position 2.

<table>
<thead>
<tr>
<th>Porphyrin Position</th>
<th>Heme a</th>
<th>Heme b</th>
<th>Heme c</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>C$<em>{17}$H$</em>{29}$O</td>
<td>CH=CH$_2$</td>
<td>CH$_3$-CH (cys)</td>
</tr>
<tr>
<td>Y</td>
<td>CH=CH$_2$</td>
<td>CH=CH$_2$</td>
<td>CH$_3$-CH (cys)</td>
</tr>
<tr>
<td>Z</td>
<td>CHO</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>
5. Cytochrome B has vinyl groups CH=CH$_2$ at position 2 & 4 and a methyl group at position B.
6. Cytochrome C has CH$_3$CH (cys) at position 2 & 4 and a methyl group at position 8.
7. Iron in Cytochromes is equatorially co-ordinated by the four pyrrole nitrogen atoms of the porphyrin ring system.
8. 5$^{th}$ & 6$^{th}$ position of iron is axially co-ordinated by different groups in the protein side chain.
9. In Cytochrome a, 5$^{th}$ & 6$^{th}$ ligands are histidine imidazole nitrogen atoms.
10. Cytochrome C has a polypeptide chain of 104 amino acids attached and wrapped around the heme group.
11. The 5$^{th}$ position of iron in Cytochrome C is co-ordinated by the imidazole ‘N’ atom of the histidine – 18.
12. The 6$^{th}$ position of Cytochrome C is attached by the thioether ‘S’ atom of the Methionine - 80.

**Transition Metal Complex In Biological Molecules:**

Metal ion complexes are used in humans for the transport and storage of oxygen as electron-transfer agents, as catalysts and as drugs. The body requires a number of minerals in order to maintain its proper functioning. The minerals are used for a variety of physiological processes such as building blood and bone, making hormones, regulating heartbeat, and more. There are two types of minerals.

Macro minerals are needed in large amounts. Trace minerals are needed in very small amounts.

Macro minerals are calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulphur. These elements contribute about 0.7% of the atoms in the human body. Absence of these elements in the body results in disease and
sometimes may result in death. These minerals are presents in greater concentration in the body.

The trace minerals are iron, manganese, copper, iodine, zinc, cobalt, fluoride, and selenium. These minerals play important nutritional role. The deficiency of these elements causes disease and it can even result in death. The excess concentration of these elements in the body will produce toxic effect.

**Biological Importance Of Iron (Fe):**

- Iron plays a central role in almost all living cells.
- Iron is used in red blood cells to carry oxygen to the tissues and is also a critical component of many metabolic proteins and enzymes.
- A healthy adult needs 10 to 18 mg of iron each day in the intake of food. The RDA of iron for men is 8 mg, for women 18 mg, and for pregnant women 27 mg.
- Iron is found in the body in the form of heme iron and non-heme iron.
- Heme iron is bound within a ring-like molecule called porphyrin. Heme iron is present in red blood cells.
- Non-heme iron such as iron-sulfur cluster proteins are used in energy production and other metabolic functions.
- Iron deficiency anemia is a condition where a lack of iron in the body leads to a reduction in the number of red blood cells.

**The Most Common Symptoms Include:**

- tiredness and lack of energy (lethargy)
- shortness of breath
- noticeable heartbeats (heart palpitations)
- a pale complexion
Less Common Symptoms Include:

- headache
- an altered sense of taste
- feeling itchy
- hair loss

Ferrous gluconate used in the treatment of anemia. It has a significant role in respiration and photosynthesis.

**Biological Importance Of Zinc (Zn):**

- Zinc plays multiple roles in the body. It is involved in many cellular metabolic processes and is used in growth and development, the immune system, neurological function, and reproduction.
- It is a trace element and an adult should have 10 to 15 mg in the body.
- It also forms a structural part of cell membranes and is a component of the zinc finger proteins, which act as transcription factors.
- Zinc also supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell.
- A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system.
- Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function.

**Biological Importance Of Magnesium (Mg):**

- Magnesium is an essential mineral and electrolyte that plays a role in many bodily processes, including energy production, bone and teeth structure, muscle function, nerve function, DNA replication and RNA and protein synthesis.
• These ions activate many of the enzymes that control the addition and removal of phosphate groups from compounds in the cell.
• Magnesium ions form the central metal ion of the chlorophyll molecule, which traps the energy from the sunlight in the process of photosynthesis and which gives green colour to the plants.
• Early symptoms of magnesium deficiency can include nausea and vomiting, loss of appetite, tiredness, and weakness.
• Although many people are not getting enough magnesium, deficiency is rare, and symptoms usually indicate an underlying health condition.
• Too much of magnesium in the body decreases muscle and nerve response, and high levels can produce local or general anesthesia and paralysis.

**Biological Importance Of Cobalt (Co):**

• Cobalt is an essential trace element that is an integral part of vitamin B12, which is essential in the metabolism of folic acid and fatty acids.
• Cobalt is involved in the production of red blood cells and is important for the proper functioning of the nervous system as it can help in creating a myelin sheath.
• It is the component of vitamin B12 includes the metabolism of carbohydrates, fats, etc.,
• Cobalt that we need is obtained from dairy products and meat.
• A lack of cobalt in the diet results in a disease called pernicious anemia which produces symptom of fatigue and general weakness.
• Too much vitamin B12, in the diet will stimulate the production of too many erythrocytes, producing a condition called polycythemia.
Biological Importance Of Molybdenum (Mo):

- Molybdenum is an essential nutrient. Its main function is in removing toxins particularly from the metabolism of sulfur containing amino acids.
- Molybdenum participates in the energy transfer reactions in the cell.
- Molybdenum helps with: energy production, by breaking down some of the amino acids; cell protection, by activating antioxidants; and waste removal, by metabolizing toxins that can be excreted in urine.
- It is necessary for the function of certain intestinal enzymes.
- The chief role of molybdenum is to activate nitrate reductase enzyme during nitrogen metabolism.
- Molybdenum participates in the energy transfer reactions in the cell.

The Role Of Sodium (Na) In Biological Activity:

- Sodium plays a key role in the regulation of blood volume, blood pressure, osmotic balance and maintains a constant pH.
- It is main base of the body.
- Sodium is an essential electrolyte that helps maintain the balance of water in and around your cells.
- It is important for proper muscle and nerve function.
- It also helps maintain stable blood pressure levels.
- Insufficient sodium in your blood is also known as hyponatremia.
- It's regulated in the body by your kidneys, and it helps control your body's fluid balance.
- A person needs 1-2 grams of sodium daily in his diet.
- It helps in maintaining the peripheral resistance of blood vessels.

The Role Of Potassium (K) In Biological Activity:

- Potassium is a mineral that plays many important roles in the body.
• Potassium is most commonly used for treating and preventing low potassium levels, treating high blood pressure, and preventing stroke.
• Potassium helps your nerves and a muscle “talk” to each other, moves nutrients into and waste out of your cells, and helps your heart function.
• Food sources of potassium include fruits (especially dried fruits), cereals, beans, milk, and vegetables.
• Average daily requirement of potassium in an adult is 4 g.
• It helps your kidneys to control your blood pressure by controlling the amount of fluid stored in your body. The more fluid, the higher your blood pressure.
• Potassium deficiency is known as hypokalemia.
• Neutralize the effect of organic acids.

The Role Of Calcium (Ca) In Biological Activity:

• Calcium is an essential element in living organisms. It plays an important role in the metabolism of nitrogen in some plants where a deficiency of calcium leads to poor absorption of nitrogen.
• Calcium is the fifth most common element in the body.
• Its major function in building and maintaining bones and teeth, calcium is important in the activity of many enzymes in the body.
• The regulation of heartbeat and clotting of blood are all dependent on calcium.
• They play an important role in signal transduction pathways, where they act as a second messenger, in neurotransmitter release from neurons, in contraction of all muscle cell types, and in fertilization.
• Almost all of the calcium in the body is stored in bone.
• Calcium is used to help blood vessels move blood throughout the body and to help release hormones and enzymes that affect almost every function in the human body.
• It gives nutrients to the development of root hairs.
• Average daily requirement of calcium in an adult is 1000 mg.
• Calcium is found in dairy products, broccoli, cabbage, kale, tofu, sardines and salmon.

**The Role Of Phosphorus (P) In Biological Activity:**

• Potassium forms the sugar-phosphate backbone of DNA and RNA. It is important for energy transfer in cells as part of ATP (adenosine triphosphate) and is found in many other biologically important molecules.
• Phosphorus also has an important role in vertebrates, whose bones and teeth contain apatite, a highly stable phosphate mineral.
• It plays an important role in how the body uses carbohydrates and fats.
• It is also needed for the body to make protein for the growth, maintenance, and repair of cells and tissues.
• Phosphorus is found in high amounts in protein foods such as milk and milk products and meat and alternatives, such as beans, lentils and nuts.
• Phosphorus also plays an important structural role in nucleic acids and cell membranes.
• It is involved in the body's energy production.
• Our body absorbs less phosphorus when calcium levels are too high, and vice versa.
• Together with calcium, phosphorus provides structure and strength.

**Electron Transfer In Iron- Sulphur Proteins:**

There are several non heme iron sulphur proteins that are involved in electron transfer reactions. They contain distinct iron- sulphur clusters composed of iron atoms, sulfhydryl group from cysteine residues and inorganic sulphur atoms. Sulphur clusters are found in a variety of metalloproteins, such as the ferredoxins, NADH dehydrogenase and etc. Iron–sulphur clusters are best known for their role
in the oxidation-reduction reactions of electron transport in mitochondria and chloroplasts. Both Complex I and Complex II of oxidative phosphorylation have multiple Fe–S clusters. There are several types of non-heme protein involved in electron transfer.

**Rubredoxin:**

1. Rubredoxins are low molecular weight iron containing proteins found in anaerobic bacteria.
2. These contain only one Fe atom; this single Fe atom is at the centre of tetrahedron of 4 cysteine sulphur atoms.
3. It does not contain inorganic sulphur atom. It is abbreviated Fe (I) so where S stands for inorganic sulphur.
4. Rubredoxins perform one-electron transfer processes. The central iron atom changes between the +2 and +3 oxidation states. In both oxidation states, the metal remains high spin, which helps to minimize structural changes.
5. This iron-sulphur protein is an electron carrier, and it is easy to distinguish its metallic centre changes, the oxidized state is red in colour (due to a ligand metal charge transfer), while the reduced state is colour less.
**Ferredoxin:**

Ferredoxins are small proteins containing iron and sulphur atoms organized as iron–sulphur clusters. These biological "capacitors" can accept or discharge electrons, with the effect of a change in the oxidation state of the iron atoms between +2 and +3. In this way, ferredoxin acts as an electron transfer agent in biological redox reactions. Ferredoxins can be classified according to the nature of their iron–sulphur clusters.

1. **Plant type:**
   1. A group of ferredoxins, originally found in chloroplast membranes, has been termed "chloroplast-type" or "plant-type".
   2. Its active center is a $[\text{Fe}_2\text{S}_2]$ cluster, where the iron atoms are tetrahedrally coordinated both by inorganic sulphur atoms and by sulfurs of four conserved cysteine (Cys) residues.
   3. In chloroplasts, $\text{Fe}_2\text{S}_2$ ferredoxins function as electron carriers in the photosynthetic electron transport chain and as electron donors to various cellular proteins, such as glutamate synthase, nitrite reductase and sulfite reductase.
   4. In hydroxylating bacterial dioxygenase systems, they serve as intermediate electron-transfer carriers between reductase flavoproteins and oxygenase.
   5. It has a bridged structure $\text{FeIIS}_2$ as shown below.
6. It acts as the electron acceptor associated with Photosystem I in photosynthesis. It accepts an electron and is reduced, giving it the capacity to pass on those electrons as part of the electron transport process.

2. **Bacterial type:**

1. A group of Fe₄S₄ ferredoxins originally found in bacteria has been called as bacterial type.
2. It consists of a cubane like clusters of 4 iron atoms, 4 inorganic sulphur atom and 4 cysteine ligands.
3. The [Fe₄S₄] ferredoxins may be further subdivided into low-potential and high-potential ferredoxins.

![Diagram of Fe₄S₄ ferredoxin](image)

**Dioxygen Binding:**

1. Hemoglobin is a tetrameric protein; mb is a monomeric protein having a polypeptide chain where as it has one heme group which consists of four subunits 2α- subunit and 2β- subunit.
2. The capacity of hemoglobin to bind the oxygen depends on the prosthetic (non-protein component) group called heme.
3. The heme group is responsible for the distinctive red colour of blood.
4. The heme group consists of an organic component and a central iron atom.
5. The organic component called porphyrin is made up of 4 pyrrole rings linked by methane bridges to form a tetrapyrrole ring.
6. The iron atom lies in the centre of the porphyrin, bonded to the four pyrrole nitrogen atoms.
7. The iron can form two additional bonds, one on each side of the heme plane. These binding sites are called 5th and 6th co-ordination sites.
8. The 5th co-ordination site is occupied by the imidazole ring of a histidine residue from the protein.
9. The deoxy hemoglobin present in the 6th co-ordination site remains unoccupied.
10. The Fe$^{2+}$-N bond length is 2.18 Å which is much greater than the mean radius in centre of the porphyrin cavity. The penta coordinated iron in the de-oxy hemoglobin has a square pyramidal geometry and it is situated about 0.8Å out of the porphyrin plane being shifted towards the histidine ring.
11. The oxygen binds to the Fe- heme at the vacant 6th position and the resulting octahedral field is sufficiently strong to transform high spin Fe(II) (radius 0.92 Å) to low spin Fe (II) (radius 0.75 Å) as a result Fe(II) radius reduced by 0.17 Å and hence Fe(II) of oxy hemoglobin moves towards the porphyrin plane and sit in the porphyrin cavity.
12. This movement of Fe (II) causes the coordinated histidine to move towards the porphyrin plane.
13. Four methyl groups, 2 vinyl groups and 2 iso propionate side chains are attached.
14. The active sites of both hemoglobin and Myoglobin contain the heme group.
Oxygen Transport And Utilisation:

1. The oxygen that we need for survival is transported from the lungs to peripheral tissues by hemoglobin.
2. Hemoglobin is densely packed in our red blood cells.
3. It is made up of 4 protein subunits which consist of a pair of α-globin chains and a pair of β-globin chains.
4. The porphyrin ring with central Fe$^{2+}$ ion is called heme.
5. One molecule of hemoglobin with its 4 heme groups is capable of binding 4 molecules of diatomic oxygen $O_2$.

6. The pigment with molecular oxygen is called oxy hemoglobin.

7. As the blood circulates to the periphery, the small amount of dissolved oxygen is consumed first by cells, organs and tissues which begin a sequential release of heme bound oxygen.

8. The pigment without oxygen is called deoxy hemoglobin.

9. During the release of oxygen, the hemoglobin tetramer undergoes some intramolecular conformational changes, called co-operativity.

10. As a result of co-operativity once the 1$^{st}$ oxygen has been released, the releasing of the 2$^{nd}$ oxygen is facilitated.

11. After the release of the 2$^{nd}$ oxygen it undergoes some conformation changes which facilitate the release of 3$^{rd}$ oxygen.

12. Co-operativity is an important phenomenon that permits the occupying and releasing of large amounts of oxygen.

**Biological Dioxygen Carriers: (Other Than Hemoglobin And Myoglobin)**

**Hemerythrin:**

1. Hemerythrin is an oligomeric non-heme protein responsible for oxygen transport in the marine invertebrates.

2. Myohemerythrin is a monomeric $O_2$ – binding protein found in the muscles of marine invertebrates.

3. Hemerythrin and Myohemerythrin are violet – pink colour in the oxygenated state and colour less in the deoxygenated state.

4. Mechanism of oxygen binding: -
   
The mechanism of dioxygen binding is unusual. Most oxygen carriers operate via formation of dioxygen complexes but Hemerythrin holds the $O_2$ as hydro peroxide (HO$_2$, or -OOH$^-$.)

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25
5. The site that binds O\textsubscript{2} consists of a pair of iron centres. The iron atoms are bound to the protein through the carboxylate side chain of a glutamate and aspartates as well as through 5 histidine residues.

6. Deoxy hemerythrin contains two high-spin ferrous ions bridged by hydroxyl group. One iron is hexacoordinate and another is pentacoordinate.

7. A hydroxyl group serves as a bridging ligand but also functions as a proton donor to the O\textsubscript{2} substrate. O\textsubscript{2} binds to the pentacoordinate Fe\textsuperscript{2+} centre at the vacant coordination site.

8. Then electrons are transferred from the ferrous ions to generate the binuclear ferric (Fe\textsuperscript{3+}) centre with bound peroxide.

9. Another oxygen containing pigment is hemocyanin. It is found in many marine species.

10. The polypeptide chain must have a molecular weight between 50000 – 75000.
11. The Cu is in +1 oxidation state in the deoxy form and it is diamagnetic in nature and so it is colour less.
12. The Cu is in +2 oxidation state in the oxy form and it is paramagnetic in nature and so it is blue in colour.
13. The dioxygen binding site appears to be a pair of copper atoms, each bound by 3 histidine residues.
14. It means oxy hemocyanin binds with oxygen because of which the Cu gets oxidized from +1 to +2. An empty cavity exists between the Cu atoms.
15. If the hemocyanin contains n number of Cu centres then it will contain n/2 of O₂ molecules. It is also called as oxo species.

**Structure And Function Of Hemoglobin:**

1. Hemoglobin is a tetramer with a molecular weight of 64,000 Daltons (64,458 g/mol) and contains 4 heme groups bound to protein chains.
2. Two of the chains are labeled as β – have 146 amino acids and the other two are labelled as α which have 141 amino acids.
3. As per Perutz mechanism the iron in deoxy heme having high spin Fe (II) configuration.
4. The radius of high spin Fe²⁺ is too large to fit within the plane of the porphyrin nitrogen atoms. Also, the Fe (II) – N bond length in high spin is 218pm which is much greater than the mean radius 205 pm of the porphyrin cavity.
5. Hence, the iron atom is forced to sit about 80 pm above the centre of the heme group towards the apically coordinated histidine ring.
6. Oxygenation of hemoglobin results in two of the heme groups moving about 100 pm towards each other while two others separate themselves by about 700 pm. It means that one αβ half of the molecule rotates 15° relative to the other half. This movement is responsible for the co-operative effects observed.
7. The deoxy form is called as ‘T’ state and that of oxy form is ‘R’ state.
8. The co-ordination of the dioxygen molecule as a sixth ligand causes spin pairing to take place on the iron atom. Since the radius of the low spin Fe (II) is about 17 pm smaller than high spin Fe (II). The Fe-N bond distance will relatively show a reduction in distance to an extent of 200 pm and hence the low spin iron atom in the porphyrin cavity. Hence, co-ordination with oxygen will therefore cause the iron atom to pull about 80 pm into the plane of the heme group.
9. Due to the movement of the iron atom into the cavity, which also pull the imidazole group of the histidine residue attached to the iron atom and the tertiary structure of the protein chain of which it takes part will be rather drastically altered.
10. The above movement of the protein chain of another heme group to react. When the fully saturated hemoglobin molecule with four O₂ molecules reaches the tissues, the whole sequence of reactions discussed above will be reversed.
Comparison Of Myoglobin (Mb) And Hemoglobin (Hb)

<table>
<thead>
<tr>
<th>Myoglobin</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. It is a heme protein with a single polypeptide chain</td>
<td>It is a heme-protein consisting of four polypeptide chain</td>
</tr>
<tr>
<td>2. It has a single O₂ binding site</td>
<td>It has four O₂ binding sites</td>
</tr>
<tr>
<td>3. Transports only O₂</td>
<td>Transports O₂, H⁺ and CO₂</td>
</tr>
<tr>
<td>4. O₂ binding is independent of H⁺ and CO₂ concentration</td>
<td>O₂ binding is controlled by H⁺ and CO₂ concentration</td>
</tr>
<tr>
<td>5. O₂ binding isotherm is hyperbolic</td>
<td>O₂ binding isotherm is sigmoidal</td>
</tr>
<tr>
<td>6. Binds O₂ even at low partial pressure in the muscle tissues</td>
<td>Binds O₂ at high partial pressure in lungs.</td>
</tr>
</tbody>
</table>

Physiology Of Myoglobin And Hemoglobin:

1. Hemoglobin and Myoglobin are two important biomolecules which are involved in oxygen transport and storage in bio systems.
2. Hemoglobin transports oxygen from lungs and the oxygen are transferred to Myoglobin for use in respiration.
Oxygen Saturation Curves Of Hemoglobin And Myoglobin

3. The oxygen binding curve can be used to describe the oxygen binding properties of Mb and Hb.
4. The y-axis describes the relative fraction of proteins that are saturated with O₂.
5. The x-axis describes the concentration of O₂ (mmHg).
6. The equilibrium constant for mb-O₂ the complexation is given by the following expression.

\[ k_{mb} = \frac{[mbO_2]}{[mb][O_2]} \]

7. The equilibrium constant for the formation of oxy hemoglobin is not as simple as that observed for myoglobin.

\[ k_{Hb} = \frac{[HbO_2]}{[Hb][O_2]}^{2.8} \]

The exponent 2.8 for oxygen results from the fact that a single hemoglobin molecule can accept 4 oxygen molecules.
8. It is quite evident from the figure that myoglobin is largely converted to oxy myoglobin even at low concentrations.
9. In hemoglobin at low concentrations, it is less oxygenated and at higher O$_2$ concentrations it is more oxygenated.

10. The presence of several bound oxygen molecules enhances the probability of the addition of more oxygen molecules.

11. If one oxygen molecule is present in general, it will dissociate more readily than from a highly oxygenated species. So, this results in a sigmoid curve for oxygenation of hemoglobin from the above figure.

12. This effect favours oxygen transport and it helps the hemoglobin gets saturated in the lungs and deoxygenated in the capillaries.
Chapter 3

Bio-Inorganics of Plants

Photosynthesis:

1. Photosynthesis is the synthesis of carbohydrates by the green organs of a plant in the presence of sunlight.
2. CO$_2$ and H$_2$O are taken from the air and soil respectively, oxygen being the by-product.

\[
\begin{align*}
6\text{CO}_2 + 12\text{H}_2\text{O} & \xrightarrow{\text{Light, Chlorophyll}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \\
6\text{O}_2 & \xrightarrow{\text{by-product}} \end{align*}
\]

3. Electrons for the reduction of CO$_2$ are obtained from water (i.e.) a reduced (NaDPH$_2$) substance is produced which later reduces CO$_2$.
4. The photochemical reaction includes two photo acts, which are called Photosystem I, Photosystem II.
5. In Photosystem I, there are different types of molecules of chlorophyll a, which absorbs photon of light.
6. On the basis of wavelength absorbed by the chlorophyll a molecule, they are classified as chl-660, chl-670, chl-680, chl-690 and p-700. (p-higher wavelength) here p-700 functions as reaction centre in which photochemical reaction takes place from where high energy electrons are released.
7. The other pigments mentioned above, function as antenna in collecting energy and this energy are transferred to reaction centre.
Process In Photosystem II:

The copy warned the Little Blind Text, that where it came from it would have been rewritten a thousand times and everything that was left from its origin would be the word.

- Electrons released from Photosystem II, reduce a substance ‘Q’. From the reduced substance ‘Q’ high energy electrons are accepted by another substance ‘B’.
- From reduced ‘B’ electrons pass on to plastoquinone. From reduced plastoquinone electrons are accepted by Cytochrome F. From reduced Cytochrome F electrons are taken by plastocyanin.
From reduced plastocyanin electrons are accepted by p-700. (Photosystem I reaction centre).

**Process In Photosystem I:**

- In the Photosystem I, by absorbing energy of photon, p-700 gets excited.
- High energy electrons released from photo system I, are accepted by the substance iron-sulphur protein complex designed at A (FeS).
- From the reduced A (FeS) these electrons are accepted by oxidised ferredoxin (Fd) by accepting this electrons Fd is reduced.
- From reduced Fd electrons are taken by FAD which is reduced to FAD\(_2\) from the reduced FAD electrons are accepted by NADP and it is reduced to NADPH\(_2\).
- The hydrogen attached to NADPH\(_2\) is used for converting CO\(_2\) to carbohydrates. This process is repeated.

**Chlorophyll And Photosynthetic Reaction Centre:**

1. In photosynthetic reaction, the reaction centre is a protein with a molecular weight of about 1,50,000.
2. The heart of the reaction centre is a pair of chlorophyll molecules referred as spectral pair.
3. The special pair on conduct with one other through the overlap of one of the pyrrole rings in each molecule.
4. In addition, an acetyl group on each molecule co-ordinates to the magnesium atom of the other.
5. All photosynthetic systems contain one or more of the green pigments called chlorophyll.
6. Chlorophyll’s are tetra pyrroles of the porphyrin family.
7. In the active sites of the chlorophyll pigment, a Mg$^{2+}$ ion is co-ordinated by the 4 pyrrole Nitrogen atoms of the porphyrin ring system at a distance of about 0.3 to 0.5 Å above the macrocycle plane.

8. The methyldiene carbon atom between the pyrrole rings III and IV is connected with the C$_6$ carbon atom of the pyrrole ring III through HC [COOCH$_3$] (C=O) moiety to form a cyclopentanone ring.

9. The long phytol chain attached to the C$_7$ carbon atom of the pyrrole ring IV.

10. The complete parent system I to IV is called porphyrin.

11. Chlorophyll absorbs light of low energy in the far-red region near 700.

12. The exact wavelength of maximum absorption depends upon the nature of substituents on chlorophyll.
Enzymes:

1. Enzymes are in general globular proteins.
2. It is a macromolecular biological catalyst. They are responsible for thousands of metabolic processes that sustain life.
3. Enzymes are generally accelerating both the rate and specify the metabolic chemical reaction.
4. Enzymes are specific to their substrate. Specificity is determined by their active site.
5. Normally increasing the temperature make molecules to react faster.
6. Biological systems are very sensitive to temperature changes.
7. Enzymes can increase the rate of a reaction without increasing temperature.
8. They do this by lowering the activation energy. They create a new reaction pathway a “Short-Cut”.
9. Enzymes controlled reactions 108 to 1011 times faster than corresponding non-enzymatic reaction.
Lock and key Hypothesis:

1. The Lock and key model of enzyme action, proposed earlier this century, proposed that the substrate was simply drawn into a closely matching cleft on the enzyme molecule.
2. Fit between the substrate and the active site of the enzyme is exact. Like a key fit into a lock precisely. This temporary structure is called the enzyme-substrate complex formed.
3. The substrate shape must be compatible with the enzymes active site in order to fit and be reacted upon.

   **The Lock and Key Mechanism**

4. The enzyme modifies the substrate. In this instance the substrate is broken down, releasing two products.

Enzymes are catalysts. Most are proteins. Enzymes bind temporarily to one or more of the reactants, the substrate of the reaction they catalyze. In doing so, they lower the amount of activation energy needed and thus speed up the reaction.
**Examples:**

Catalase: It catalyzes the decomposition of hydrogen peroxide into water and oxygen.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

**Vitamin B\(_{12}\):**

1. Vitamin B\(_{12}\) is a water-soluble vitamin involved in the metabolism of every cell of the human body. It is a co-factor in DNA synthesis and in both fatty acid and amino acid metabolism.
2. Vitamin B\(_{12}\) is also known as cobalamin, cyanocobalamin, methyl cobalamin etc., used for all compounds containing a corrin nucleus.
3. The structure of vitamin B\(_{12}\) is based on a corrin ring, which is nearly planar, macrocyclic ring like the porphyrin system found in hemes, chlorophylls and cytochromes.
4. A cobalt atom lies at the centre of corrin ring, the cobalt is in oxidation state +3, four of the six co-ordination sites are provided by the corrin ring, and a fifth by a dimethyl benzimidazole group.
5. In the sixth co-ordination site, where the biological activity centre of vitamin B\(_{12}\) is a variable, being a cyano group (-CN), a hydroxyl group (-OH), a methyl group (-CH\(_3\)) or a 5’-deoxyadenosyl group.
6. Vitamin B$_{12}$ is biologically active in only three forms, adenosyl cobalamin, hydroxocobalamin and methyl cobalamin.

**B$_{12}$ co-enzymes:**

1. Vitamin B$_{12}$ is biologically inactive and its active forms are known as B$_{12}$co-enzymes or co-factors which play important roles in the essential enzymatic reactions related to nucleic acid, protein and lipid synthesis.
2. Vitamin B$_{12}$ is produced by microorganism (bacteria/ fungi) and this is only vitamin which contains metal (cobalt).
3. B$_{12}$ co-enzyme contains an adenosyl group linked to the cobalt centre by a direct C-Co bond, which indicates the presence of a metal-carbon bond in the biological systems.
4. The B$_{12}$ coenzyme is considered to be one of the most stable $\sigma$-organocobalt bonds.
5. A macrocyclic ligand system in the vitamin B$_{12}$ that actually influences and modifies the properties of cobalt significantly, enabling it to form a highly stable Co–C bond.
6. The known B₁₂ coenzymes/cofactors are alkyl cobalamins, consisting of a cobalt complex of tetrapyrrole macrocyclic ligand (corrin ring) with a pendent nucleotide (intra-molecularly bound 5,6-dimethylbenzimidazole) which occupies the five coordination site of an octahedral Co(III), and the sixth position being occupied by different R groups in different cofactors, methyl cobalamin (R = CH₃) and coenzyme B₁₂.

The corrin ring system in vitamin B₁₂ coenzymes is roughly planar, and the short side chains extend above the corrin ring plane while the long side chains extend below the plane of ring.

![Vitamin B₁₂ Co-enzyme](image)

**Vitamin B₁₂ Co-enzyme**

**Metallothionein:**

1. Metallothioneins belong to the group of intracellular cysteine-rich, metal binding proteins that have been found in bacteria, plants and humans.
2. Metallothioneins are low molecular weights proteins that bind heavy metals, such as zinc, copper, cadmium, nickel, etc.,

3. They have a high content of cysteine residues that bind the metal ions through clusters of thiolate bonds.

4. Metallothioneins plays a role in the protection against metal toxicity and oxidative stress, and is involved in zinc and copper regulation.

5. Metallothioneins likely participate in the uptake, transport, and regulation of zinc in biological systems.

6. MT binds three Zn (II) ions in its beta domain and four in the alpha domain. Cysteine is a sulphur-containing amino acid, hence the name "thionein".

7. Metallothionein also carries zinc ions (signals) from one part of the cell to another. When zinc enters a cell, it can be picked up by thionein (which thus becomes "metallothionein") and carried to another part of the cell where it is released to another organelle or protein.

8. In this way thionein and metallothionein becomes a key component of the zinc signaling system in cells. This system is particularly important in the brain, where zinc signaling is prominent both between and within nerve cells.

9. It also seems to be important for the regulation of the tumor suppressor protein p53.

10. Metallothionein also plays a role in hematopoietic cell differentiation and proliferation, as well as prevention of apoptosis of early differentiated cells.

11. Induced MT levels were adversely associated with sensitivity to etoposide-induced apoptosis, signifying that MT is a potential negative controller of apoptosis.

12. This protein functions in primary metal storage, transport, and detoxification. More specifically, yeast MT stores copper so therefore protects the cell against copper toxicity by tightly chelating copper ions.
13. Metallothionein gene expression is induced by a high variety of stimuli, as metal exposure, oxidative stress, glucocorticoids, Vitamin D, hydric stress, fasting, exercise, etc.

14. The level of the response to these inducers depends on the MT gene. MT genes present in their promoter’s specific sequences for the regulation of the expression, elements as metal response elements (MRE), glucocorticoids response elements (GRE), GC-rich boxes, basal level elements (BLE), and thyroid response elements (TRE).

**Metalloproteins:**

Proteins which contain a metal atom such as iron, copper, magnesium, zinc etc as an integral part of the structure are called metalloproteins. There are two types of metalloproteins:

1. **Heme Proteins:**

These are metalloproteins containing the heme group. Heme is the prosthetic group consisting of a porphyrin ring chelated to an iron atom. The heme proteins are coloured and hence called chromo proteins.

**Examples:**

Cytochromes (electron carriers), haemoglobin (oxygen carrier), myoglobin (oxygen storage site), ferritin (iron storage site)

2. **Blue copper proteins:**

These are metalloproteins which contain copper as the active site. Examples are stellacyanin, platocyanin and azurin. These are Cu (II) complexes which functions as electron transfer redox systems. Each of these has pseudo tetrahedral (between tetrahedral and square-planar) geometry. The Cu (I)/ Cu (II) centre in
these systems is ideally adapted for electron exchange as it involves no change in spin state.

The blue copper proteins occur in some bacteria known as cyanobacteria (also called blue-green algae). Some of these species are free-living, others live in close association with plants. Some of them convert the atmospheric nitrogen into nitrogen compounds hence used as biofertilizers.

**Metalloenzymes:**

1. Enzymes incorporated with metal ions in their structures are called metalloenzymes. Zn$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Mn$^{2+}$, Mo$^{2+}$ etc are metal ions found in metalloenzymes.

2. The metal ions are bonded at or near the active site of the enzyme and enhance the activity of the enzyme. In the absence of such metal ions, the enzymes are rendered inactive.

**Examples:**

1. Carboxy peptidase is a metalloenzymes containing zinc. It occurs in the pancreas of mammals and catalyses the hydrolysis of the peptide bond at the carbonyl end of the peptide chain.

2. Cytochrome oxidase, ascorbic acid oxidase and tyrosine are copper containing enzymes. They catalyse oxidation reactions.

3. Nitrogenases are metalloenzymes which promote the fixation of atmospheric nitrogen. They are found to contain Mo and Fe.

**Carboxy Peptidase:**

1. Carboxy peptidase is a pancreatic enzyme that cleaves carboxyl terminal amino acid from a peptide chain by hydrolysing the amide linkage.
2. The zinc ion is bound in a distorted tetrahedral environment by two histidine nitrogen atoms and one atom of oxygen from a glutamic carbonyl group.

3. The fourth co-ordination site is free to accept a pair of electrons from the donor atom in the substrate to be cleaved.

4. Co-ordination of water to Zn$^{2+}$ may enhance the rate of equilibrium between H$_2$O and the OH$^-$ nucleophile.

5. Zn$^{2+}$ may serve as a Lewis acid catalyst by co-ordination to peptide carbonyl group and thereby reduce the electron density at its carbon atom and promotes hydrolysis.

6. The carbonyl oxygen atom of the peptide linkage that is to be broken has replaced the water molecule in the co-ordination sphere of the zinc ion.

7. On addition, a nearby hydrophobic pocket envelops the organic group of the amino acid to be cleaved and those amino acids with aromatic side groups react most readily.
8. The argentine side chain moves about 200 pm closer to the carboxylate group of the substrate; the Phenolic group or tyrosine comes with the H- bonding distance of the imido group of c-terminal acid, a shift of 200 pm.

9. The hydrogen bonding to the free carboxyl group by argentine and the amide linkage by tyrosine this not only holds the substrate to the enzyme but helps to break the N-C bond.

10. Nucleophilic displacement of the amide group by an attacking carboxylate group from a glutamate could from an anhydride link to the remainder of the peptide chain.

11. Hydrolysis of this anhydride could then complete the cycle and regenerate the original enzyme. More likely, the glutamate acts indirectly by polarising a H₂O molecule that attacks the amide linkage.

12. This illustrates the basic key and lock theory first proposed by Fischer in which the enzyme and substrate fit each other sterically.

13. There is a good evidence at the enzyme also encourages the reaction by placing strain on the bond to be broken.

14. From the evidence of spectroscopic studies in enzymes containing metal ions that, unlike Zn²⁺ shows d-d transitions. The spectrum of the enzymes containing such a metal ion provides information on the micro symmetry of the site of the metal.

15. For example, Co²⁺ can replace Zn²⁺ and the enzyme retains the activity. The spectrum of carboxy peptidase A (Co⁴⁺) is irregular and has a high absorptivity, indicating that a regular tetrahedron is not present.

16. The distortion is due to the metal in the enzyme is peculiarly poised for action and that this lowers the energy of the transition state.

**Carbonic Anhydrase:**

1. Carbonic anhydrase is a Zn – enzyme. This enzyme occurs in red blood cells.
2. It catalyses hydration of CO$_2$ below pH 7 and dehydration of the bicarbonate ion according to the following reaction.

$$\text{OH}^- + \text{CO}_2 \rightarrow \text{HCO}_3^-$$

3. It has one zinc atom per molecule; it has co-ordinated to three histidine residues His 94, His 96 and His 119. The fourth co-ordination site is binded with a H$_2$O molecule or hydroxide ion.

4. The active site contains other amino acids that may function through H-bonding, proton transfer etc.,

5. The relative binding power of the zinc ion towards halide ions is reversed in the enzyme I$^-$>Br$^-$>Cl$^-$>F$^-$ compared with the free Zn$^{2+}$ ions as F$^-$.  

6. This softening effect on the zinc by the apoenzyme it not that the soft ligand CN$^-$ is bound equally well by the free ion as by the complex.

7. NO$_3^-$, CNO$^-$ and N$_3^-$ none of which is known for exceptional softness are bound with exceptional strength. They are isoelectronic, isostructural with the reactants and products of the enzyme reaction CO$_2$, CO$_3^{2-}$, HCO$_3^-$ respectively.

8. The structure of the enzyme molecule to form a pocket of about 450pm long next to the zinc ion, perhaps containing an additional positive centre, to stabilize ions of approximate size.

9. In some mechanisms, the CO$_2$ co-ordinated directly to the Zn- atom, this is highly not possible. It catalyzes hydration of CO$_2$ below pH 7 and dehydration of the bicarbonate ion according to the following reaction.

$$\text{OH}^- + \text{CO}_2 \rightarrow \text{HCO}_3^-$$

10. The IR asymmetric stretching frequency for CO$_2$ is found to be 2343.5 cm$^{-1}$ for the free molecule, which shows good interaction of one O$_2$ atom and not the other.

11. The visible spectrum of Co$^{2+}$ substituted enzyme shows very small shift upon binding CO$_2$, again incompatible with strong O$_2$-metal interactions.
12. The Zn atom is thought to be considerably more acidic in carbonic anhydrase than in carboxy peptidase.

Mechanism Of Reversible Hydration Of CO₂ To Carbonic Acid:

1. The substitution of a third, neutral and basic histidine in place of glutamate anion contributes the greater acidity.
2. The three histidines are pulled back making the Zn more electronegative and more acidic towards the fourth position.
3. This polarises an attached H$_2$O molecule, to the point of loss of an H$^+$ ion to form a co-ordinated hydroxo group. The above diagram shows the pathway of the reversible hydration of CO$_2$ to carbonic acid (HCO$_3^-$).

4. It is a closed loop; it may be carried out either clockwise to hydrate CO$_2$ or anti-clockwise to release CO$_2$ from ion from HCO$_3^-$ blood to the lungs.

5. The rates of the forward and reverse reactions in the hydration of CO$_2$ equilibrium increases as the pH are raised.

6. The ligand H$_2$O is rapidly interconverting with a zinc-co-ordinated OH$^-$ ligand that acts as the nucleophile towards CO$_2$.

7. The mechanism involves the attack on the carbon atom of CO$_2$ by the rapid reaction of OH$^-$ ligand on Zn$^{2+}$ followed by the formation of a transient five co-ordinate Zn$^{2+}$ ion in which a carbanato oxygen from HCO$_3^-$ co-ordinates to the Zn$^{2+}$ ion.

8. After rearrangement, the HCO$_3^-$ ligand is displaced by H$_2$O. Deprotonation of Zn$^{2+}$ co-ordinated H$_2$O then regenerates the OH$^-$ ligand, which can attack another CO$_2$ molecule, with the repetition of the catalytic cycle.

9. The above mechanism, relates a part on the x-ray and crystal studies proved that the structure of carbonic anhydrase, which indicates the presence of a hydrophobic nature adjacent to the site of CO$_2$. This rapid hydration or dehydration by carbonic anhydrase appears to occur at a site near Zn$^{2+}$.

10. Ligands that can co-ordinate to an active centre in an enzyme and prevent co-ordination by the substrate will tend to inhibit the action of that enzyme.

**Nitrogen Fixation:**

1. Nitrogen fixation is the process by which nitrogen is taken from its relatively inert molecular form (N$_2$) in the atmosphere and converted into nitrogen compounds useful for other chemical processes.

2. Fixation of atmospheric nitrogen is an important step in the nitrogen cycle, providing nitrogen for plant nutrition.
3. Nitrogen fixation readily occurs in various bacteria blue green algae, yeasts and in symbiotic bacteria-legume associations under mild conditions. However, N\textsubscript{2} resists ordinary chemical attack, even under stringent conditions.

4. Nitrogen is so insensitive to chemical reactions that it has been characterised as inert as a noble gas. The very large N≡N bond energy of 945 KJ/mol makes the activation energy very large.

5. Inspite of the fact that the overall enthalpy of formation of ammonia is exothermic by about 50 KJ/mol, the Haber process requires about 20 mpa pressure and 500° c temperature even in the pressure of best catalyst.

6. The conversion of free atmospheric nitrogen into useful nitrogenous compounds by artificial or natural methods is called fixation of nitrogen. Nitrogen present in these nitrogenous compounds is called fixed or combined nitrogen.

**Fixation of Atmospheric Nitrogen:**

1. **Fixation of N\textsubscript{2} as NH\textsubscript{3} by Haber’s Process:**

   A mixture of nitrogen (manufactured by liquefaction of air) and hydrogen in the ratio 1:3 is compressed to a presence of 200-500 atmospheres and is passed over a catalyst (finely divided iron + molybdenum) heated to about 550° c. This forms the Haber’s process for the manufacture of ammonia which then can be converted into ammonium salts by treatment with suitable acids.

   \[ \text{N}_2 + 3\text{H}_2 \xrightarrow{\text{Catalyst, High press}} 2\text{NH}_3 \]

2. **Fixation of N\textsubscript{2} as HNO\textsubscript{3} by Ostwald’s Process:**

   NH\textsubscript{3} manufactured by Haber’s process is oxidised to nitric oxide (NO) by passing a mixture of NH\textsubscript{3} (1 volume) and air (8 volumes) over heated Pt gauze at 1070K.
NO combines with more of O₂ to give nitrogen dioxide (NO₂) which when absorbed in water in the presence of excess of air, gives HNO₃.

\[
\begin{align*}
4 \text{NH}_3 (1 \text{ vol}) + 5\text{O}_2 (8 \text{ vol}) & \xrightarrow{\text{Pt gauze}} 4 \text{NO} + 6\text{H}_2\text{O} \\
2\text{NO} + \text{O}_2 (\text{Excess}) & \rightarrow 2 \text{NO}_2 \\
4 \text{NO}_2 + 2\text{H}_2\text{O} + \text{O}_2 (\text{Excess}) & \rightarrow 4 \text{HNO}_3
\end{align*}
\]

3. Fixation of N₂ as HNO₃ by Birkland-Eyde Process:

Under the influence of high-tension electric arc where the temperature is high, nitrogen of the air combines with oxygen to form nitric oxide. It combines with more of oxygen to form nitrogen peroxide. This may be absorbed in water in presence of excess of air to give nitric acid which may be used for the manufacture of nitrogenous fertilizer.

\[
\begin{align*}
\text{N}_2 + \text{O}_2 & \rightarrow 2\text{NO} \text{ (Nitric oxide)} \\
2\text{NO} + \text{O}_2 & \rightarrow 2 \text{NO}_2 \text{ (Nitrogen peroxide)} \\
4 \text{NO}_2 + 2\text{H}_2\text{O} + \text{O}_2 & \rightarrow 4 \text{HNO}_3
\end{align*}
\]

4. Fixation of N₂ as Ammonium Salts and Nitrates:

NH₃ obtained by Haber’s process and HNO₃ obtained by Ostwald’s process and Brikland-Eyde process can be used for the preparation of ammonium salts and nitrates which are used as fertilizers.

5. Fixation of Nitrogen as Calcium Cyanamide:

Nitrogen gas obtained by the evaporation of liquid air is passed over calcium carbide heated to 800-1000° c. A mixture of calcium cyanamide and carbon is obtained which is extensively used as a fertiliser under the name of nitrolim.
6. **Fixation of Nitrogen as Nitrides:**

Nitrogen combines with magnesium and aluminium at the temperature to give nitrides which are employed as a source of ammonia. These nitrides are decomposed by $\text{H}_2\text{O}$ and $\text{NH}_3$ is evolved.

\[
\begin{align*}
3 \text{Mg} + \text{N}_2 & \rightarrow \text{Mg}_3\text{N}_2 \\
\text{Magnesium nitride} \\
2\text{Al} + \text{N}_2 & \rightarrow 2 \text{AlN} \\
\text{Aluminium nitride} \\
\text{AlN} + 3\text{H}_2\text{O} & \rightarrow \text{Al(OH)}_3 + \text{NH}_3
\end{align*}
\]

**In Vitro Nitrogen Fixation:**

1. The molecular nitrogen was capable of forming stable complexes with transition metals led to extensive investigation of the possibility of fixation of nitrogen via such complexes.
2. Titanium (II) alkoxide form dinitrogen complexes which are then reduced with subsequent release of $\text{NH}_3$ or hydrazine.

\[
\begin{align*}
\text{Ti (OR)}_4 + 2\text{e} & \rightarrow \text{Ti (OR)}_2 + 2\text{RO}^- \\
\text{Ti (OR)}_2 + \text{N}_2 & \rightarrow [\text{Ti (OR)}_2\text{N}_2] \\
[\text{Ti (OR)}_2\text{N}_2] + 4\text{e} & \rightarrow [\text{Ti (OR)}_2\text{N}_2]^{4-} \\
[\text{Ti (OR)}_2\text{N}_2]^{4-} + 4\text{H}^+ & \rightarrow \text{N}_2\text{H}_4 + \text{Ti (OR)}_2 \\
[\text{Ti (OR)}_2\text{N}_2]^{6-} + 2\text{e} & \rightarrow [\text{Ti (OR)}_2\text{N}_2]^{6-} \\
[\text{Ti (OR)}_2\text{N}_2]^{6-} + 6\text{H}^+ & \rightarrow 2\text{NH}_3 + \text{Ti (OR)}_2
\end{align*}
\]
3. Under certain conditions, the starting material is regenerated making the reaction catalytic. However, after a few cycles, the starting materials are depleted.

4. These reactions are not commercially compatible to Haber process for synthesizing ammonia but are useful in the synthesis of hydrazine and other organic nitrogen compounds.

5. Certain phosphine complexes of Mo and W containing dinitrogen readily yield ammonia in acidic media in presence of Grignard reagents as reducing agents at room temperature and atmospheric pressure.

\[
\begin{align*}
[\text{MoCl}_3(\text{thf})_3] + 3e^- + N_2 + \text{excess dpe} & \rightarrow [\text{Mo}(N_2)_2(\text{dpe})_2] + 3\text{Cl}^- \\
[\text{Mo}(N_2)_2(\text{dpe})_2] + 6\text{H}^+ & \rightarrow 2\text{NH}_3 + \text{N}_2 + \text{Mo}^{VI} \text{ Products}
\end{align*}
\]

thf--- tetrahydrofuran; dpe --- 1,2- bis (diphenyl phosphino) ethane

6. This reaction is important as (i) it models the in vivo nitrogenase enzyme which employs Mo (ii) it provides insight into the development of useful catalysts for the industrial fixation of nitrogen.

**In Vivo Nitrogen Fixation:**

1. Clostridium pasteurianum, klebsiella pneumoniae, azobacteria vinelandi and rhizobium (living in root nodules of legumes) are the bacteria which fix molecular nitrogen in vivo.

2. The active enzyme is nitrogenase. It differs from species to species. However, the various enzymes are very similar.

3. The nitrogenase consists of two distinct oxygen sensitive proteins, both of which are essential for nitrogen fixation.
4. One protein has a molecular weight of 57000-73000 containing Fe₄S₄ cluster. The other protein has molecular weight 22000-25000 containing two Mo atoms, 20-30 Fe atoms and 20-30 S atoms.

5. Fe-S clusters present in both the proteins act as redox centres. Nitrogen is bound to Mo in the second protein, C₂H₂ is also very effectively bound and reduced by the enzyme.

6. This enzyme also reduces N₂H₄ to NH₃, thus suggesting that N₂H₄ may be an intermediate in the mechanism.

7. The electrons are supplied by pyruvate through ferredoxin to the Fe-S clusters of nitrogenase. Two Mo (III) atoms cycling through Mo (VI) provide the six electrons necessary for the reduction of dinitrogen.

**Transition Metals:**

Transition metals are important to the chemistry of living systems, the most familiar examples being iron, cobalt, copper, and molybdenum. The transition metals are usually present as trace elements in organisms, with zinc and iron being most abundant. These metals are used as protein cofactors and signaling
molecules. Iron is by far the most widespread and important transition metal that has a function in living systems; proteins containing iron participate in two main processes, oxygen transport and electron transfer (i.e., oxidation–reduction) reactions. There are also a number of substances that act to store and transport iron itself.

Cobalt is understood to be an essential trace element in animal nutrition, the only detailed chemical knowledge of its biochemical action has to do with vitamin B_{12} and related coenzymes. These molecules contain one atom of cobalt bound in a macrocyclic ring (i.e., one consisting of many atoms) called corrin, which is similar to a porphyrin ring. Copper is found in both plants and animals, and numerous copper-containing proteins have been isolated.

Metal ions are usually required to promote and stabilize functionally active or native conformations of nucleic acids, as well as to mediate nucleic acid-protein interactions. However, certain metal ions can also cause structural transformation of nucleic acids and induce their chemical modification and cleavage. Transition metal ions play an important role in catalysing a number of chemical and biological reactions. The reactivity of metal complex can be modulated at high levels by simply changing the metal ions and their oxidation states. The successful synthesis and application of novel mono and multinuclear metal complexes can have a great impact on all areas of chemistry viz., organic, medicinal and biological chemistry. In recent times transition metal complexes have attracted considerable interest due to their efficient interactions with DNA under various physiological conditions for different applications of metal complexes in nucleic acid chemistry.
Chapter 5

Applications of Bio-Inorganics in Medicine

Anticancer Activity of Platinum Complexes:

Platinum complexes are used to treat approximately half of all patients receiving cancer chemotherapy. Cisplatin was the first platinum compound discovered to have anticancer activity. Following its introduction into the clinical management of cancer, two other platinum drugs received widespread regulatory approval, carboplatin and oxaliplatin. All three compounds exert their cytotoxic effects by coordinating to DNA where they inhibit both replication and transcription and trigger a cell death pathway. This focuses mainly on cisplatin, the prototypical platinum anticancer drug, and its properties highlight the general features of platinum drugs as a class.

Metal chelation apparently plays a definite role in the cause and treatment of cancer. The anticancer activity of metal complexes is attributed to the transfer of metal ion from the complex to the viruses associated with cancer. Some platinum complexes show anti-tumour activity. These include Cis-Pt (NH\textsubscript{3})\textsubscript{2} Cl\textsubscript{2}, Cis-Pt (NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{4}, Pt (en) Cl\textsubscript{4} of which Cis-Pt (NH\textsubscript{3}) Cl\textsubscript{2} is the most effective and more active than the corresponding bromo and iodo complexes. Cis-Pt (NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}, commonly known as Cis-platin, has a wide spectrum of anticancer activity. Trans-platin has no effect on Escherichia coli.

Cis-Pt (NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2} is a very effective antitumor agent causing regression of both fast and slow growing cancers. The mechanism is not clear. In the high Cl\textsuperscript{-} concentration of extra cellular fluids, replacement of Cl\textsuperscript{-} by H\textsubscript{2}O molecule is suppressed. However, after passing into the cell, where the Cl\textsuperscript{-} concentration is about 1/30 \textsuperscript{th} of that outside the cell, the following reactions takes place.
The diaquo species reacts to form Cis-Pt (NH$_3$)$_2$ bridges between the N- atoms of DNA bases, producing mainly intrastrand DNA cross links rather than DNA-protein cross- links. Bridging of diaquo species occurs preferentially to DNA, which contains high proportions of quinine and cytosine. Studies under biological conditions establish that bridging occurs to quinine, cytosine and adenine but not to thymine.

Cis-platin must hydrolyse in the right place. If it hydrolysers in the blood before it gets in to the chromosomes within the cell, it will be more likely to react with a non-target species.

A related chemotherapeutic agent, diamine (1,1-cyclobutanedicarboxylato) platinum (II) is known as Cis-platin. This compound undergoes reaction more slowly and produces less severe side effects than Cis-platin.
Some platinum complexes inhibit the growth of cancerous cells. Therefore, these complexes are used as anticancer drugs in cancer therapy.

**Examples:**

1. 

\[
\begin{array}{c}
\text{Cl} \\
\text{Pt} \\
\text{Cl} \\
\text{NH}_3 \\
\text{NH}_3 \\
\end{array}
\]

Cis-diamminedichloro platinum II

Cis-platin

2. 

\[
\begin{array}{c}
\text{Cl} \\
\text{Pt} \\
\text{Cl} \\
\text{NH}_2 \\
\text{NH}_2 \\
\text{CH}_2 \\
\text{CH}_2 \\
\end{array}
\]

Cis-ethylenediamminedichloro platinum II

3. 

\[
\begin{array}{c}
\text{O} \\
\text{C} \\
\text{O} \\
\text{Pt} \\
\text{NH}_3 \\
\text{NH}_3 \\
\end{array}
\]

Cis-oxalatodiammineplatinum II

4. 

\[
\begin{array}{c}
\text{Cl} \\
\text{Pt} \\
\text{Cl} \\
\text{NH}_2\text{C}_6\text{H}_5 \\
\text{NH}_2\text{C}_6\text{H}_5 \\
\end{array}
\]

Cis- dichlorodiphenylamine platinum II
5.

\[
\begin{array}{c}
\text{Cis-diammine-1,1-cyclobutane dicarboxylate platinum II} \\
\text{Carboplatin}
\end{array}
\]

6.

\[
\begin{array}{c}
\text{Cis-ammine (aniline)dichloroplatinum II}
\end{array}
\]

7.

\[
\begin{array}{c}
\text{Cis-diammine (pyridine) chloroplatinum II}
\end{array}
\]

**Mode of Action:**

The exact mode of action of the platinum complexes is not known. It is only the cis isomer, which is active at low concentrations, not the trans isomer. Therefore, it is presumed that the two cis groups in the complex are replaced by some other groups in the cancer cell, forming a chelate ring. Such an association helps destroying cancerous cells. The replacement of the groups in the trans position by a chelating reagent is not easy and hence, the trans isomers of platinum complexes do not have therapeutic property.

**Copper Complexes:**

Some of the copper complexes are found to have anti-inflammatory activities. Therefore, these complexes are used for the treatment of arthritis. Examples:
1. Copper salicylate (permalon)
2. Sodium-3- (allylcuprothiouredo)-1-benzoate (cupralene)
3. Bis- (8-hydroxyquinolene) bis- (diethyl amine sulphonate) copper II (dicuprene)
4. Cu (II)-D-penicillamine (DPA)
5. Cu (II)-L-tyrosinate
6. Cu (II)-aspirinate

**Mode of Action:**

Lack of superoxide dismutase (SOD), a copper containing enzyme may cause arthritis. The copper complexes increase the concentration of SOD and display anti-arthritic activities.
Chapter 6

Advanced Tools

Docking:

Schematic illustration of docking a small molecule ligand (green) to a protein target (black) producing a stable complex.

In molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

The associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced.
Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes.

**Molecular Docking:**

Molecular Docking is a valuable tool in structural biology and computer–aided drug design. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The chief goal of ligand–protein docking is to predict the predominant binding modes of a ligand with a protein of known three–dimensional structure. It also predicts the strength of the binding, the energy of the complex; the types of signal produced and calculate the binding affinity between the two molecules using scoring functions. Successful docking methods do search for the high–dimensional spaces effectively and use a scoring function that correctly ranks the dockings.

To provide information about the interactions between the human cytochrome protein and the novel compounds theoretically, docking studies were carried out using the Schrödinger software. Characterization of the binding behaviour plays an important role in the rational design of drugs as well as to elucidate the fundamental biochemical processes.

Molecular docking can be divided into two separate sections.

**1. Search Algorithm:**

The algorithm should create an optimum number of configurations that include the experimentally — determined binding modes. The following are the various algorithms used for docking analysis.
• Molecular dynamics
• Monte Carlo methods
• Genetic algorithms
• Fragment–based methods
• Point complementary methods
• Distance geometry methods
• Systematic searches

2. Scoring Function:

These are the mathematical methods used to predict the strength of the non–covalent interaction called as binding affinity, between the two molecules, after they have been docked. Scoring functions have also been developed to predict the strength of other types of intermolecular interactions, for example, between the two proteins or between the protein and the DNA or protein and the drug. These configurations are evaluated using scoring functions to distinguish the experimental binding modes from all other modes explored through the searching algorithm.

Types of Docking:

The following are the chief methods used for docking

Lock and Key or Rigid Docking:

In rigid docking, both the internal geometry of the receptor and the ligand is kept fixed and the docking is performed.

Induced Fit or Flexible Docking: An enumeration on the rotations of one of the molecules (usually smaller one) is performed. For every rotation, the surface cell occupancy and the energy are calculated; later the most optimum pose is selected.
Software’s Available for Molecular Docking:

- SCHRÖDINGER
- DOCK
- MOLEGro VIRTUAL DOCKER
- AUTODOCK
- iGemDock
- BETADOCK
Chapter 7

Further Reading

For Further Reading:

1. U. Saityanarayana, Essentials of Biochemistry, Books and Allied (P) Ltd.,
About the Author

Dr. A. Therasa Alphonsa obtained Ph.D., in Molecular Docking. She extensively teaches Bio-Inorganic Chemistry course to the UG and PG students of Annamalai University and Government Arts College, Chidambaram. Her research interests are mainly in Organic Synthesis, Drug Docking and recently in Material Science. She has published many international research papers and books for her credit.